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# Reduction of bacterial adhesion on Ag-TiO<sub>2</sub> coatings

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## ABSTRACT

Ag-TiO<sub>2</sub> nano-composite coatings showed the synergistic effect on the anti-bacterial activity. However, little was known on the mechanism of the enhanced anti-bacterial effect. In this paper, a range of Ag-TiO<sub>2</sub> nano-composite coating with different TiO<sub>2</sub> contents were prepared on titanium substrate using an electroless deposition technique and the antibacterial performance of the coatings were evaluated with *E. coli* and *S. aureus*. This work demonstrated for the first time that the incorporation of TiO<sub>2</sub> nano-particles into Ag matrix not only significantly promoted the Ag ion release, but also significantly increased the electron donor surface energy, which lead to the enhanced antibacterial effect of the Ag-TiO<sub>2</sub> coatings.

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## 1. Introduction

Titanium (Ti) and its alloys are widely used in both orthopedic and dental implants due to their excellent mechanical strength, resistance to corrosion and biocompatibility. However these implants are highly susceptible to biofilm formation, leading to implant-associated infections [1]. Both silver and titanium dioxide (TiO<sub>2</sub>) have been known as broad-spectrum bactericides for a long time. The antibacterial efficiency of silver coatings mainly depends on the release rate of silver ions from the coatings [2], while the TiO<sub>2</sub> photocatalysts generate strong oxidizing power for the observed damaging cell properties when illuminated with UV light [3,4]. Recently it has been demonstrated that the Ag-TiO<sub>2</sub> coatings show the synergistic effect on the antibacterial activity [5,6]. However, the mechanism are still not well understood. The Ag-TiO<sub>2</sub> coatings are usually prepared by sol-gel [7] or by magnetron sputtering [8] or by CVD [9]. Electroless plating is a cost-effective coating technique with inherent uniform coating thickness and excellent coating adhesion strength to the substrate [10]. To the best of our knowledge, there is no report on the preparation of Ag-TiO<sub>2</sub> nanocomposite coatings using the electroless plating technique. The aim of the present work is to investigate the mechanism of the enhanced antibacterial effect of the Ag-TiO<sub>2</sub> coatings prepared by the electroless plating technique.

## 2. Materials and methods

### 2.1. Preparation of Ag-TiO<sub>2</sub> coatings

Pure Ti disks (10 mm diameter) were used as substrates. UV irradiation was carried out on the both sides of the disks at 385 nm for 2 hs to activate the inert Ti surfaces, following by the immersion of the disks into coupling agent solution for 2hs in order to create the reactive surfaces as described previously [11]. The TiO<sub>2</sub> powder was brought from Aldrich, with particle size of 25 nm. The plating bath composition mainly included AgNO<sub>3</sub> 2–7 g/l; NaOH 1.5–5 g/l; NH<sub>3</sub>·H<sub>2</sub>O 100–150 ml/l; C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> 2–7 g/l and TiO<sub>2</sub> 0.1–2 g/l. A range of Ag-TiO<sub>2</sub> coatings with the different TiO<sub>2</sub> concentrations (0.1, 0.3, 0.5, 1.5 and 2 g/l) were prepared by an electroless plating technique at room temperature under dark and stirring (40 rpm) conditions. Ag coating was also prepared for comparison.

### 2.2. Characterization of Ag-TiO<sub>2</sub> coatings

The surface morphology of the coatings were observed by scanning electron microscopy (SEM) (S-4800, Japan). The elemental compositions were determined by Energy Dispersive X-ray analysis (EDX) (EMAX350, Japan). The release of Ag ions from the coatings were determined by inductively coupled plasma atomic emission spectrometry ICP-AES (Varian 710-ES, USA). The Ag coating or Ag-TiO<sub>2</sub> coating was immersed in sealed tank containing 25 ml of PBS and kept at 37 °C at 150 rpm, and then the concentration of Ag ions was measured at different immersion time. The contact

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angle on the coatings after exposed UV light was measured using the sessile drop method with a Dataphysics OCA-20 contact angle analyzer with distilled water (W), diiodomethane (Di) and ethylene glycol (EG) as the test liquids, respectively. The surface energy components of the coatings, including Lifshitz-van der Waals (LW) apolar ( $\gamma_{2-}^{LW}$ ), electron donor ( $\gamma_{2-}$ ) and electron acceptor ( $\gamma_{2+}$ ) were calculated using the van Oss approach [12].

### 2.3. Bacterial adhesion

*E. coli* (ATCC 8739) and *S. aureus* (ATCC 6538) were used for bacterial adhesion assays. The bacterial suspension with a  $10^7$  CFU/ml concentration for each type of bacteria was prepared using the method described previously [10]. For bacterial adhesion assays, five replicate samples of each coating were immersed in a glass tank containing 25 ml of the bacterial suspension at 37 °C for 1 hr, 24 hr, 3 days and 7 days, respectively. Then the total number of adhered bacteria for the each sample were determined using a viable plate counting method [10].

## 3. Results and discussion

### 3.1. Surface characterizations

In this study, 5 types of Ag-TiO<sub>2</sub> coatings with different TiO<sub>2</sub> concentrations (0.1, 0.3, 0.5, 1.5 and 2 g/l) were prepared. The corresponding TiO<sub>2</sub> contents in the coatings which were 2.40, 3.05, 4.82, 9.56 and 12.60 wt% respectively, were determined by EDX analysis based on Ti content in the coatings (Table 1). Fig. 1a shows the morphology of Ag-TiO<sub>2</sub> coating (TiO<sub>2</sub> 9.56 wt%) with typical polygon rounded hillock units for Ag structure. The TiO<sub>2</sub> particles with sizes in the range of 25 nm–50 nm (white dots) were homogeneously incorporated in the Ag matrix. Fig. 1b shows the corre-

sponding EDX image of the Ag-TiO<sub>2</sub> coating. The element composition for Ti, O and Ag was 5.73 wt%, 17.18 wt% and 77.09 wt%, respectively.

The ICP-AES measurements indicated that the Ag ion release rates from all the Ag-TiO<sub>2</sub> coatings were much higher than that from the Ag coating. Fig. 2 shows typical comparison results between the Ag-TiO<sub>2</sub> coating (TiO<sub>2</sub> 9.56 wt%) and the Ag coating. Clearly, the incorporation of TiO<sub>2</sub> nano-particles into Ag coating promoted Ag ion release.

Table 1 shows the contact angle and surface energy of the coatings used in this investigation, including pure Ti, Ag coating and Ag-TiO<sub>2</sub> coatings with different TiO<sub>2</sub> contents. The results indicated that, in general, the electron donor surface energy ( $\gamma_{2-}$ ) of the Ag-TiO<sub>2</sub> coatings increased with increasing TiO<sub>2</sub> contents in the coatings.

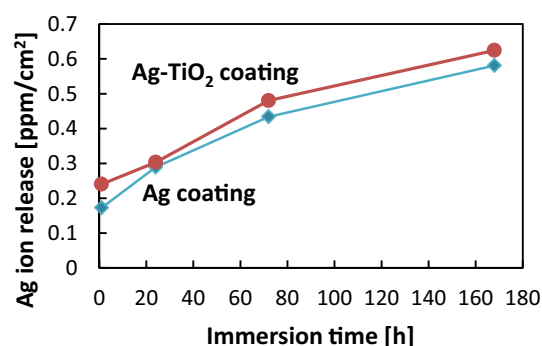
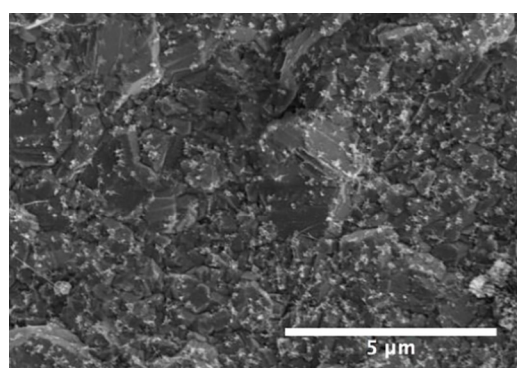


Fig. 2. Comparison of Ag ion release rate from the Ag-TiO<sub>2</sub> coating (TiO<sub>2</sub> 9.56 wt%) and the Ag coating.

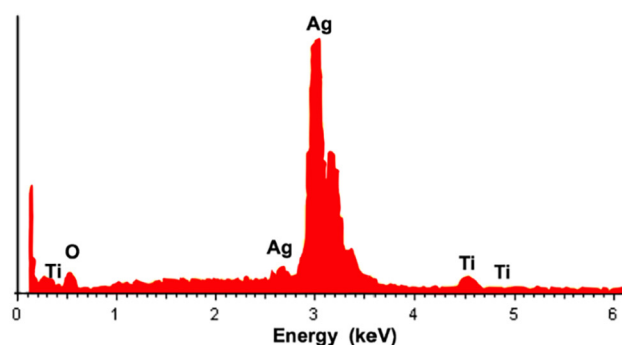
Table 1

Contact angle and surface energy components of coating samples.

Sample	TiO <sub>2</sub> content (wt. %)	Contact angle, $\theta$ (deg)			Surface free energy (mJ/m <sup>2</sup> )			
		$\theta^W$	$\theta^{Di}$	$\theta^{EG}$	$\gamma_{2-}^{LW}$	$\gamma_{2-}^+$	$\gamma_{2-}^-$	$\gamma_{2-}^{TOT}$
Ti	–	79.2 ± 1.5	60.0 ± 0.3	37.1 ± 0.5	28.58	2.92	3.64	35.09
Ag	–	73.7 ± 1.2	48.5 ± 0.6	46.9 ± 1.1	35.11	0.36	9.93	38.90
Ag-TiO <sub>2</sub> 1	2.40	62.8 ± 2.2	42.4 ± 1.0	38.9 ± 1.2	38.38	0.24	18.33	42.61
Ag-TiO <sub>2</sub> 2	3.05	68.1 ± 3.1	46.3 ± 1.4	47.8 ± 2.1	36.31	0.09	16.15	38.78
Ag-TiO <sub>2</sub> 3	4.83	70.7 ± 2.5	54.0 ± 0.9	57.0 ± 0.6	32.02	0.01	18.12	32.89
Ag-TiO <sub>2</sub> 4	9.56	61.4 ± 0.5	45.9 ± 0.2	53.3 ± 1.5	36.53	0.05	28.04	38.91
Ag-TiO <sub>2</sub> 5	12.69	57.8 ± 1.4	63.3 ± 0.5	32.0 ± 0.3	26.68	2.06	23.42	40.56



(a)



(b)

Fig. 1. Typical (a) SEM and (b) EDX images of Ag-TiO<sub>2</sub> coating with TiO<sub>2</sub> 9.56 wt%.

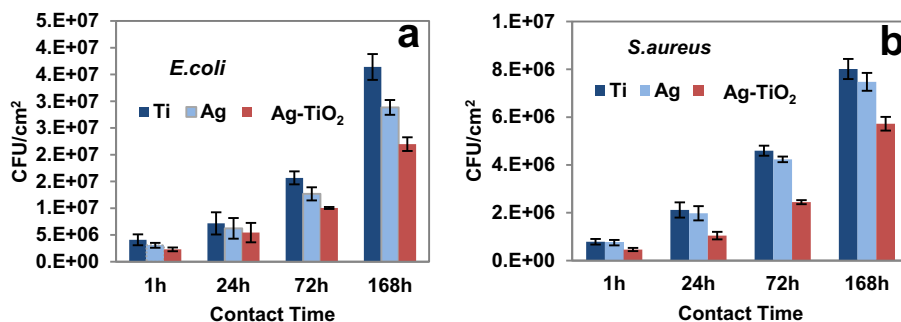


Fig. 3. Comparison of anti-bacterial performance of Ti surface, Ag coating and Ag-TiO<sub>2</sub> coating with (a) *E. coli* and (b) *S. aureus* (N = 15, error bars are standard Error).

### 3.2. Bacterial adhesion assays

The results show that the number of adhered bacteria increased with increasing contact time for all the coatings. The Ag-TiO<sub>2</sub> coatings performed best against bacterial adhesion compared with Ti surface and Ag coating at different contact time. Fig. 3 shows typical evaluation results with the Ag-TiO<sub>2</sub> coating (TiO<sub>2</sub> 9.56 wt%). The Ag-TiO<sub>2</sub> coating reduced the adhesion of *E. coli* by up to 43.5% and 24.5%, respectively as compared with the Ti surface and the Ag coating (Fig. 3a). The Ag-TiO<sub>2</sub> coating reduced the adhesion of *S. aureus* by up to 50.7% and 47.2%, respectively as compared with the Ti surface and the Ag coating (Fig. 3b).

## 4. Discussion

It is well known that the antibacterial efficiency of silver based coatings increases with increasing the release rate of Ag ions [2]. The results indicated that the Ag ion release rates from all the Ag-TiO<sub>2</sub> coatings were much higher than that from the Ag coating (Fig. 2). This finding explains the mechanism of the synergistic effect of Ag-TiO<sub>2</sub> coatings or why the Ag-TiO<sub>2</sub> coatings performed better than the Ag coating against bacterial adhesion. In general bacteria are negatively charged and the bacteria are repelled from the surface by similar charge, when closing to the surface. It was reported that the surface charge relates to the surface energy components of electron donor ( $\gamma_{2-}$ ). The higher  $\gamma_{2-}$  value, the more negatively charged the surface [11]. Table 1 clearly indicates that the  $\gamma_{2-}$  value of the Ag-TiO<sub>2</sub> coatings significantly increased due to the incorporation of TiO<sub>2</sub> nano-particles into Ag coatings. This means the incorporation of TiO<sub>2</sub> nano-particles increases the negative charge of the Ag-TiO<sub>2</sub> coatings. As both the *E. coli* and *S. aureus* are negatively charged, this further explains why the Ag-TiO<sub>2</sub> coatings are repellent to the bacteria and performed much better than the Ag coating or Ti surface against bacterial adhesion.

## 5. Conclusions

The incorporation of TiO<sub>2</sub> nano-particles into Ag matrix not only significantly promoted the Ag ion release, but also significantly increased the electron donor ( $\gamma_{2-}$ ) surface energy or negative surface charge of the coatings, which lead to the enhanced antibacterial effect of the Ag-TiO<sub>2</sub> coatings.

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## References

- [1] B.S. Necula, J.P.T.M. van Leeuwen, L.E. Fratila-Apachitei, S.A.J. Zaat, I. Apachitei, J. Duszczek, In vitro cytotoxicity evaluation of porous TiO<sub>2</sub>-Ag antibacterial coatings for human fetal osteoblasts, *Acta Biomaterialia* 8 (2012) 4191–4197.
- [2] W.R. Fordham, S. Redmond, A. Westerland, E.G. Cortes, C. Walker, C. Gallagher, C.J. Medina, F. Waechter, C. Lunk, R.F. Ostrum, C.A. Caputo, J.D. Hettinger, R.R. Krchnavek, Silver as a bactericidal coating for biomedical implants, *Surf. Coat. Tech.* 253 (2014) 52–57.
- [3] M.D. Lorenzetti, S.N. Biglino, S. Kobe, Photoinduced properties, of nanocrystalline TiO<sub>2</sub>-anatase coating on Ti-based bone implants, *Mater. Sci. Eng. C Mater. Biol. Appl.* 37 (2014) 390–398.
- [4] R. Aminedi, G. Wadhwa, N. Das, B. Pal, Shape-dependent bactericidal activity of TiO<sub>2</sub> for the killing of Gram-negative bacteria *Agrobacterium Tumefaciens* under UV torch irradiation, *Environ. Sci. Pollut. Res.* 20 (2013) 6521–6530.
- [5] A.L. Motlagh, S. Bastani, M.M. Hashemi, Investigation of synergistic effect of nano sized Ag/TiO<sub>2</sub> particles on antibacterial, physical and mechanical properties of UV-curable clear coatings by experimental design, *Prog. Org. Coat.* 77 (2014) 502–511.
- [6] N.T.T. Hoang, N.V. Suc, T.V. Nguyen, Bactericidal activities and synergistic effects of Ag-TiO<sub>2</sub> and Ag-TiO<sub>2</sub>-SiO<sub>2</sub> nanomaterials under UV-C and dark conditions, *Int. J. Nanotechnol.* 12 (2015) 367–379.
- [7] E. Albert, P.A. Albouy, A. Ayral, P. Basa, G. Csik, N. Nagy, S. Roualdes, V. Rouessac, G. Safran, A. Suhajda, Z. Zolnai, Z. Horvolgyi, Antibacterial properties of Ag-TiO<sub>2</sub> composite sol-gel coatings, *RSC Adv.* 5 (2015) 59070–59081.
- [8] P. Navabpour, S. Ostovarpour, J. Hampshire, P. Kelly, J. Verran, K. Cooke, The effect of process parameters on the structure, photocatalytic and self-cleaning properties of TiO<sub>2</sub> and Ag-TiO<sub>2</sub> coatings deposited using reactive magnetron sputtering, *Thin Solid Films* 571 (2014) 78–83.
- [9] J. Mungkalasiri, L. Bedel, F. Emieux, J. Dore, F.N.R. Renaud, C. Sarantopoulos, F. Maury, CVD elaboration of nanostructured TiO<sub>2</sub>-Ag thin films with efficient antibacterial properties, *Chem. Vapor Depos.* 16 (2010) 35–41.
- [10] C. Liu, Q. Zhao, Influence of surface-energy components of Ni-P-TiO<sub>2</sub>-PTFE nanocomposite coatings on bacterial adhesion, *Langmuir* 27 (2011) 9512–9519.
- [11] R. Guo, G. Yin, X. Sha, Q. Zhao, L. Wei, H. Wang, The significant adhesion enhancement of Ag-polytetrafluoroethylene antibacterial coatings by using of molecular bridge, *Appl. Surf. Sci.* 341 (2015) 13–18.
- [12] C.J. van Oss, *Interfacial Forces in Aqueous Media*, Marcel Dekker, New York, 1994.